REMARKS

Claims 45-47, 49-55 and 57-78 are all the claims pending in the application.

Claims 73-78 have been withdrawn from consideration as being directed to a non-elected invention.

Claim 45 has been amended incorporate the recitation of claim 48 and to be directed to a cell expressing recombinase Cre in a FLP-dependent manner in the presence of FLP, which is provided by a helper adenovirus expressing recombinase FLP, and being a host cell for producing, when infected with the helper adenovirus, a helper-dependent recombinant adenovirus vector that expresses a desired protein.

That is, claim 45 has been amended so as to more specifically define the host cell that is used, along with a helper adenovirus expressing recombinase FLP, in a helper-dependent adenovirus vector system for constructing a helper-dependent recombinant adenovirus vector expressing a desired protein. In this system, a helper adenovirus functions as both a helper virus providing proteins necessary for the replication of the helper-dependent recombinant adenovirus vector expressing the desired protein and a helper virus expressing recombinant FLP, which allows the host cell to express recombinase Cre in a FLP-dependent manner.

Support for this system can be found in the specification at page 13, line 21 to page 14, line 4.

Similarly, claim 55 has been amended so as to expressly recite that recombinase FLP is introduced by the use of a helper adenovirus expressing recombinase FLP. As a result, claim 55 has been deleted.

Claims 47, 51, 55, 60 and 65 have been editorially amended for clarity.

Claims 52 and 61 have been amended to delete the word "desired" as it lacks antecedent basis.

Accordingly, no question of new matter arises and entry of the amendment is requested, respectfully.

II. Detailed Action

A) Elections/Restrictions (Office Action, page 2)

The Examiner asserted that newly submitted method claims 73-78 are directed to an invention that is independent and distinct from the invention originally claimed, namely a cell. Accordingly, the Examiner has withdrawn claims 73-78 from consideration as being directed to a non-elected invention.

Because the method claims are limited to the subject matter claimed in the product claims, Applicants request that the method claims be rejoined upon indication that the product claims are allowable.

B) Claim Rejections - 35 U.S.C. § 112 (Office Action, page 3)

1) Claims 45-72 were rejected under 35 U.S.C. 112, second paragraph as being indefinite.

a) As to claim 45, the Examiner asserted that it was not clear whether the recombinant adenovirus vector that produces the desired protein also produces the helper virus. The Examiner further asserted that it appeared that the host cell produces a recombinant adenovirus vector rather than a desired protein.

Claim 45 has been amended to clarify the structure of the genome of the host cell and to clarify that when infected with the helper virus, the host cell produces a helper virus dependent recombinant adenovirus vector that expresses a desired protein.

b) With respect to claim 47, the Examiner asserted that it was not clear why the claim recites that the cell "derives from" a particular cell line.

Claim 47 has been amended to state that the cell is a human fetus kidney-derived cell line 293 cell. It is clear from claim 45 that claim 47 is reciting the cell before the modifications recited in claim 45.

c) The Examiner asserted that claims 51, 60 and 65, were unclear because it appears that the cell comprises the poly (A) sequence rather than the stuffer sequence. The Examiner suggested revised language.

Claims 51, 60 and 65 have been amended according to the Examiner's suggestions.

The Examiner also asked whether the "desired protein" mentioned in claims 51, 60 and 65 is the same as the desired protein in claim 45.

The desired protein in claims 51, 60 and 65 is a protein that acts to suppress the expression of the Cre gene, and is not the "desired protein" of claim 45. Claims 51, 60 and 65 have been amended to identify this protein by its function.

In view of the above, the Examiner is requested to reconsider and remove the rejection based on indefiniteness.

2) Claim 45 was rejected under 35 U.S.C. § 112, second paragraph as being incomplete for omitting essential elements. The Examiner asserted that the claim omits the elements that define the Cre function.

The recitation of claim 48 has been incorporated into claim 45 and claim 48 has been canceled.

Accordingly, the Examiner is requested, respectfully, to reconsider and remove this rejection.

D) Claim Rejections - 35 U.S.C. § 103 (Office Action, page 4)

Claims 45-72 were rejected under 35 U.S.C. 103(a) as being unpatentable over Hardy (WO 97/32481, previously cited) and Akagi, et al. (NAR 1997, vol. 25, No. 9, pages 1766-1773, newly cited) in view of Wahl, et al. (WO 92/15694, previously cited).

The Examiner's position was that Hardy teaches a cell that expresses Cre and that is used to make helper dependant adenovirus vectors, and that Hardy teaches that there is the possibility that if Cre is expressed all the time there could be a negative selective pressure towards what the Cre is acting on. The Examiner pointed out that Hardy uses a 293 cell line that does not produce Cre in parts 1 and 2 of the method to produce recombinant adenovirus and uses a 293 cell line that expresses Cre in part 3 of the method. The Examiner recognized that Hardy does not teach recombinase FLP as a way to regulate expression of Cre.

According to the Examiner Akagi, *et al.* teach that adenovirus virus vectors expressing a recombinase can be used to control the expression of specific genes that are integrated into the chromosomes of the cells and that Cre and FLP are known recombinases.

Finally, Wahl, *et al.* was cited as allegedly teaching recombinase FLP in a site specific gene activation system using FLP recombinase to control expression of a gene product in a manner that depends on expression of recombinase FLP.

The Examiner concluded that one of ordinary skill in the art wanting to control expression of recombinase Cre would readily modify the cells of Hardy to contain the adenovirus vectors of Akagi, et al. and in view of Wahl, et al. or Akagi, et al. would use FLP to control expression of Cre.

As seen from amended claim 45, the cell of the present invention expresses recombinase Cre in a FLP-dependent manner in the presence of FLP, which is provided by a helper adenovirus expressing recombinase FLP, and is therefore a host cell for producing, when infected with the helper adenovirus, a helper-dependent recombinant adenovirus vector which expresses a desired protein.

That is, the cell of the present invention is used as a host cell, along with a helper adenovirus expressing recombinase FLP, in a helper-dependent adenovirus vector system. In this system, a helper adenovirus functions as both a helper virus providing the helper-dependent recombinant adenovirus vector with proteins necessary for the replication thereof and a helper virus expressing recombinant FLP, which is served for the cell of the present invention to express recombinase Cre in a FLP-dependent manner.

Therefore, in this system, the helper adenovirus expresses recombinase FLP to thereby act on the cell of the present invention. Thus, the action of recombinase FLP on the cell of the present invention cuts off from the cell a stuffer sequence that is located between two recognition sequences of recombinase FLP in the cell. As a result, the cell of the present invention expresses recombinase Cre. In other words, the cell of the present invention expresses recombinase Cre in a FLP-dependent manner through the presence of recombinase FLP, which is provided by the helper adenovirus. Hence, in the present invention, the expression of recombinase Cre is

controlled by recombinase FLP. Therefore, recombinase Cre can be transiently expressed when desired, and the permanent expression of recombinase Cre can be avoided, thereby reducing the cytotoxicity of recombinase Cre against the cells.

In this system, recombinase Cre expressed by the cell of the present invention then acts on the helper adenovirus having a packaging sequence between two recognition sequences (i.e., loxP sequence) of recombinase Cre. As a result, the packaging sequence is cut off from the helper adenovirus, whereby the helper adenovirus is inhibited from replicating. On the other hand, the helper adenovirus provides the helper-dependent recombinant adenovirus vector expressing the desired protein with proteins necessary for the replication thereof. Thus, a helper-dependent recombinant adenovirus vector expressing the desired protein can be effectively produced.

As is clear from the above, this system enables the effective production of the recombinant adenovirus vector expressing the desired protein, while avoiding the contamination of the recombinant adenovirus vector with the helper adenovirus by effectively inhibiting the replication of the helper adenovirus by the action of recombinase Cre which is produced by the cell of the present invention in a FLP-dependent manner in the presence of recombinase FLP.

Consequently, the cell of the present invention is used effectively in this system.

Comparison of Present Invention with References

(1) As asserted by the Examiner in the outstanding Office Action at page 5, lines 5 to 9, Hardy discloses that it is not advantageous to grow recombinant virus in a cell expressing Cre all the time. Further, Hardy uses a 293 cell line that does not produce Cre in parts 1 and 2 of the

method to produce recombinant adenovirus and use a 293 cell line that expresses Cre in the part 3 of the method.

However, as the Examiner admitted in the Office Action at page 6, line 10, Hardy does not teach recombinase FLP as a way to regulate expression of Cre.

In this regard, the Examiner has asserted in the office Action at page 6, lines 11 to 13 that Akagi *et al.* teach that adenovirus vectors expressing a recombinase can be used to control the expression of specific genes that are integrated into the chromosomes of cells and that both Cre and FLP are known recombinases.

Consequently, the Examiner has concluded in the Office Action at page 8, lines 4 to 6 that it would be *prima facie* obvious to control the expression of Cre in a Cre expressing cell as taught by Hardy with the adenovirus of Akagi *et al.* with the expectation of success in making a cell that produces Cre in a FLP dependent manner.

However, none of the cited references teaches or suggests the use of the FLP system in the control of expression of Cre, namely the control of expression of recombinase by another recombinase as achieved by the present invention. A person skilled in the art could not have conceived of controlling expression of recombinase by another recombinase. This is because there has been no need in the art to go to the trouble to control expression of recombinase by another recombinase, and there has been no work in the art combining two recombinases in such a way. This is discussed below in more detail.

As shown in Table I attached to the Amendment filed on December 29, 2003, in the method of Hardy, the host cell is changed to a Cre-expressing cell only in the final step because Cre acts only in the final step, i.e., step 3,. Therefore, there is no motivation in Hardy for a

person skilled in the art to go to the trouble to control Cre by another recombinase as is done in the present invention. More specifically, Hardy does not motivate a person skilled in the art to form the cell of amended claim 45 of the present invention which expresses recombinase Cre in a FLP-dependent manner in the presence of recombinase FLP provided by the helper adenovirus.

The cell of amended claim 45 has a gene encoding recombinase Cre along with a stuffer sequence between two recognition sequences for recombinase FLP to inhibit expression of the recombinase Cre. When a helper adenovirus expressing recombinase FLP is introduced into the cell of the present invention, recombinase FLP is expressed and acts on the cell of the present invention to cut off the stuffer sequence from the cell. As a result, the cell of the present invention can express recombinase Cre.

That is, the cell of the present invention uses the complicated constructions as discussed above, and can express recombinase Cre in an FLP-dependent manner in the presence of recombinase FLP.

Clearly, there is no motivation in any of the cited references for a person skilled in the art to use such a complicated cell as described in amended claim 45. (By analogy, when going to San Francisco from Tokyo, nobody goes to San Francisco via New York. Everybody goes directly to San Francisco.)

Thus, it appears that the Examiner's rejection was made in hindsight, by reference to the present invention. That is, only after being made aware of the present invention does the Examiner arrive at the present invention from the teachings of the cited references. Thus, the Examiner's rejection is legally improper.

Furthermore, as mentioned above, even if a person skilled in the art wanted to control the expression of recombinase Cre by recombinase FLP, he would not know how to specifically do so, because none of the cited references teaches specifically the claimed manner of control.

Nonetheless, the Examiner appears to assert in the Office Action at page 7, lines 16 o 20 that a person skilled in the art could control expression of recombinase Cre by using a recombinant adenovirus vector expressing recombinase FLP based on the teaching of Akagi *at al.*, even though Hardy teaches nothing of FLP.

However, a person skilled in the art would not know how to specifically control expression of recombinase Cre by recombinase FLP to form the cell of the present invention, which is used for producing a helper-dependent adenovirus vector expressing a desired protein. In the present invention, there is no use of a recombinant adenovirus vector expressing recombinase FLP. Instead, the present invention uses a helper adenovirus expressing recombinase FLP and having packaging sequences between two recognition sequences (i.e., loxP sequence) of recombinase Cre. Thus, in the present invention, the helper adenovirus provides recombinase FLP, which controls expression of recombinase Cre in the cell of the present invention. On the other hand, recombinase Cre expressed in the cell of the present invention acts on the helper adenovirus to cut off the packaging sequences to render the helper adenovirus unreplicatable. Thus, the present invention can avoid contamination by the helper adenovirus of the recombinant adenovirus vector expressing a desired protein.

In this regard, neither Hardy nor Akagi *et al.* teaches or suggests the production of a helper-dependent adenovirus vector expressing a desired protein by using the cell expressing recombinase Cre in a FLP-dependent manner, as achieved by the present invention.

Noticeably and importantly, Akagi *et al.* teach merely the excitation of a gene between two loxP by the reaction of loxP and reocombinase Cre. Akagi *et al.* teach nothing whatsoever of the control on expression of recombinase Cre by recombinase FLP.

In view of the foregoing, none of the cited references teaches or suggests the control of expression of recombinase by recombinase FLP.

Consequently, none of the cited references teaches or even suggests the cell of amended claim 45.

(2) As mentioned in detail herein above, the cell of the present invention expresses recombinase Cre in a FLP-dependent manner in the presence of FLP, which is provided by a helper adenovirus expressing recombinase FLP, and is therefore a host cell for producing, when infected with the helper adenovirus, a helper-dependent recombinant adenovirus vector that expresses a desired protein.

The cell of the present invention is used as a host cell, along with a helper adenovirus expressing recombinase FLP, in a helper-dependent adenovirus vector system.

In this system, a helper adenovirus functions as both a helper virus providing the helper-dependent recombinant adenovirus vector with sequences necessary for the replication thereof and a helper virus expressing recombinant FLP, which allows the cell of the present invention to express recombinase Cre in a FLP-dependent manner.

Therefore, in this system, the helper adenovirus expresses recombinase FLP to thereby act on the cell of the present invention. Thus, the action of recombinase FLP on the cell of the present invention cuts off from the cell a stuffer sequence that is located between two recognition sequences of recombinase FLP in the cell. As a result, the cell of the present invention expresses

recombinase Cre, and as a result, recombinase Cre can be transiently expressed when desired. Thus the permanent expression of recombinase Cre can be avoided to thereby reduce the cytotoxicity of recombinase Cre against cells.

In this system, recombinase Cre expressed by the cell of the present invention acts on the helper adenovirus having a packaging sequence between two recognition sequences (loxP sequence) of recombinase Cre. Thus, the packaging sequence is cut off from the helper adenovirus, whereby the helper adenovirus is inhibited from replicating. On the other hand, the helper adenovirus provides the helper-dependent recombinant adenovirus vector expressing the desired protein with proteins necessary for the replication thereof.

Thus, the helper-dependent recombinant adenovirus vector expressing the desired protein can replicate due to the proteins necessary for the replication provided by the helper adenovirus, and can be effectively produced.

As is clear from the above, this system enables the effective production of the recombinant adenovirus vector expressing the desired protein, while avoiding contamination by the helper adenovirus into the objective recombinant adenovirus vector by effectively inhibiting the replication of the helper adenovirus by the action of recombinase Cre which is produced by the cell of the present invention in a FLP-dependent manner in the presence of recombinase FLP.

Accordingly, the Examiner is requested, respectfully, to reconsider and remove the rejection.

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the

Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

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